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Serosurvey of Selected Infectious Agents in Free-ranging and Captive Lions (Panthera leo) in Zimbabwe

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1 Zusammenfassung

Für die *Conservation Medicine* bei Löwen sind Kenntnisse über das Vorkommen von Infektionserregern von zunehmender Bedeutung. Da nur wenige Daten über Simbabwe zur Verfügung stehen, war Ziel es dieser Studie, das Vorkommen und die Zusammenhänge von Antikörpern gegen verschiedene Infektionserreger in Serumproben von Löwen aus Simbabwe nachzuweisen und die Resultate mit anderen Regionen Afrikas (Tansania, Kenia, Südafrika, Namibia) zu vergleichen.
Die Serumproben (n=72) wurden von 1993 bis 2003 von wilden und in Gefangenschaft lebenden Löwen entnommen. Untersucht werden Antikörper gegen das Feline Herpesvirus (FHV), Calicivirus (FCV), Parvovirus (FPV), Coronavirus (FCoV), Immundeffizienzvirus (FIV), Leukämievirus (FeLV), Canines Staupevirus (CDV), *Toxoplasma gondii*, *Ehrlichia canis* und *Anaplasma phagozytophilum*.
Bei den wilden Löwen (n=43) kommen Antikörper gegen FHV und *T.gondii* sehr häufig vor (FHV, 81%; *T.gondii*, 86%). Basierend auf der Kreuzreaktivität von FIV in Westernblots wird der Schluss gezogen, dass das Löwenlentivirus bei wilden Löwen in Zimbabwe endemisch vorkommt, da 52% der Proben positive Resultate geben. Ein Kontakt mit FPV kann in 23% der Proben festgestellt werden. Tiefe Prävalenzen finden wir bei FCoV (7%), *A.phagazytophilum* (14%) und bei CDV (7%). Keine Evidenz gibt es für das Vorkommen von E. canis, FCV und FeLV.
Die Prävalenzen variieren zwischen den verschiedenen Regionen des südlichen und östlichen Afrikas, daraus folgt, dass das Umplatzieren von Tieren sehr überlegt vorgenommen werden muss.

2 Abstract

Information about the exposure of lions (*Panthera leo*) to infectious agents is of increasing importance for conservation medicine. Only limited data are available for Zimbabwe. Thus, the aims of this study were to investigate the prevalences and interrelationships of antibodies to several infectious agents in serum samples from lions in Zimbabwe and to compare these results to those reported from other regions in Africa (Tanzania, Kenya, South Africa, Namibia). Serum samples (n = 72) collected between 1990 and 2003 from free-ranging and captive lions were analyzed regarding to antibodies to feline herpesvirus (FHV), calicivirus (FCV), parvovirus (FPV), coronavirus (FCoV), immunodeficiency virus (FIV), leukaemia virus (FeLV), canine

distemper virus (CDV), *Toxoplasma gondii*, *Ehrlichia canis*, and *Anaplasma phagocytophilum*. Among the free-ranging lions (n = 43) antibodies to FHV and to *T. gondii* were highly prevalent (FHV, 81%; *T. gondii*, 86%). Based on the crossreactivity with FIV in Western blots, it was concluded that a lion lentivirus is endemic in free-ranging lions in Zimbabwe as 52% of the samples were considered positive. Exposure to FPV could be detected in 23% of the samples. We found low prevalences of antibodies to FCoV (7%), *A. phagocytophilum* (14%), and CDV (7%) and no evidence for exposure to *E. canis,* FCV and FeLV. Prevalences vary in different regions of southern and eastern Africa, thus any translocation of animals must be considered carefully.

3 Introduction

During recent years there has been an increasing interest in infectious diseases in free-ranging lions (*Panthera leo)*. Besides zoological and ethological aspects, profound knowledge of the infectious status is important. The loss of habitat might be the most important factor for decreasing population sizes. However, for conservation purposes, the understanding of infectious agents also plays an important role, especially while planning translocations of seropositive animals to seronegative lion populations. Transmission of several infectious agents may be more efficient in lions than in other species of wild felids because lions are social animals living in prides. This fact may raise the susceptibility of lions to epidemic diseases like the canine distemper virus CDV outbreak that struck the Serengeti lions in late 1993 - early 1994 (23). The pathogenetic role of some of the infectious agents for lions is still unclear. In contrast, there is profound knowledge about the agent's different transmission modes, symptoms and durations of infections in domestic cats (13) as these diseases are of veterinary interest. In the domestic cat, feline calicivirus (FCV) causes inflammatory lesions in the oral cavity (gingivitis, vesicles on the tongue) affection of the joints and rarely a hemorrhagic-like fever (20). Feline parvovirus (FPV) is responsible for diarrhea and bone marrow depression often leading to leucopenia especially in young cats. Feline herpes virus (FHV) is responsible for rhinitis and conjunctivitis; infected cats remain latently infected for prolonged time. Feline coronavirus (FCoV) usually does not cause disease but occasionally mutates and induces fatal infectious peritonitis. Feline immunodeficiency virus (FIV) leads to loss of $CD4^+$ lymphocytes and to immunodeficiency eventually resulting in death from opportunistic infections. Feline leukemia virus (FeLV) induces viremia in a portion of infected cats resulting in immunosuppression, anemia or lymphoma/leukemia. In the present study we investigated the prevalence of antibodies to the above viral feline infections. In addition, antibodies to canine distemper (CDV), to *Ehrlichia canis (E. canis)*, to *Anaplasma phagocytophilum (A. phagocytophilum)* and to *Toxoplasma gondii (T. gondii*). CDV is usually important for dogs where it causes immunosuppression, affections of the respiratory tract and the central nervous system. Occasionally, CDV crosses species and induces severe epidemic outbreaks with high mortality in large felids (23). *E. canis* and *A. phagocytophilum* are rickettsial infections transmitted by tick vectors infecting blood leukocytes. *E. canis* can cause severe clinical signs in dogs while *A. phagocytophilum* affects several species and

usually causes transient fever and depression. *Toxoplasma gondii* is a protozoan parasite infecting a wide range of intermediate hosts resulting in the formation of extraintestinal tissue cysts of long persistence. In definitive hosts, the cat and other felid species, parasite development includes an intestinal phase with subsequent excretion of parasite oocysts and the formation of tissue cysts as in intermediate hosts. The detection of anti-*Toxoplasma* antibodies identifies animals carrying tissue cysts and was performed in this study as *T. gondii* infection was reported to be of clinical importance in lions (18) and to be widespread in southern Africa (4).

In East Africa, FCV, FPV, FHV, FCoV, and FIV infections were reported to be all highly prevalent (10). In contrast, in the Kruger National Park, FCV and FCoV infections were not observed (27) and interestingly, in Etosha with exception of FHV, the other viral infections were largely absent (29).
As there is only limited information available on infectious diseases in lions in Zimbabwe in southern Africa (Fig. 1), the present study was undertaken to determine the seroprevalences of the above mentioned infections and to compare the results with those of other countries in Africa.

4 Material and Methods

4.1 Animals and samples

Between 1990 and 2003, 72 serum samples were collected from 43 free-ranging and from 29 captive lions (*Panthera leo*) by a team of field researchers under the supervision of one of us (C.F.). The numbers of lions sampled per year were 4 for 1990, 4, 7, 8, 3, 6, 5, 24, 10 and 1 for the years of 1995 to 2003, respectively. Information on sex and age was available from 51 and 50 animals, respectively. The free-ranging lions inhabited areas next to Lake Kariba, Mola (n=1), Bumi Hills (n=11), Fothergill Is. (n=3), Tashinga (n=2), also Chiredzi, Malilangwe (n=11), Zambezi Valley, Mana Pools (n=11), and Hwange National Park, Main Camp (n=2), Sinamatella (n=1) (Fig. 1). No information was available about the origin of one free-ranging lion. Fothergill Island and Tashinga are in the Matusadona National Park, on the shores of Lake Kariba, and the lions sampled there are part of a contiguous population, as are the lions of Mola and Bumi Hills.

The sera were stored at -20°C until examination. Samples were sent for analysis by air courier to our laboratory. Shipment of samples was in accordance with a CITES import permit.

All sera were examined for the presence of antibodies to FHV, FCV, FPV, FCoV, FeLV, FIV, CDV, *E. canis*, *A. phagocytophilum* and *T. gondii*. FIV-Western blot testing was performed with all sera with the exception of 1 due to its limited quantity. Results were analyzed with respect to habitat, sex and age of the sampled animals as well as the year of blood collection.

4.2 Serological assays

4.2.1 Detection of antibodies to FHV, FCV, FPV

Antibodies to FHV, FCV and FPV were detected by immunofluorescence assay (IFA), as described previously (10). The IFA tests were carried out using slides masked with Teflon coating leaving open 10 dots where the antigen-containing cells were attached.

Preparation of the virus-infected cells: briefly, CRFK cell monolayers were grown in 75 cm^2 cell culture flasks in RPMI 1640 medium (Sigma, Buchs SG, Switzerland) with 10% fetal calf serum (Bio concept, Allschwil, Switzerland), 1% L glutamine 200 mM (Invitrogen, Basel, Switzerland) and 1% Antibiotic/Antimycotic Solution (PEN G, SPT,

AMB; Invitrogen) at 37°C, 5% CO_2. When the monolayers showed 90% confluence they were infected by the different viruses and harvested when the cytopathic effect (the cells rounded up and detached from the bottom of the flask) reached 60% of the cells. The detached cells were removed with the medium and washed twice with Hanks balanced salt solution (HBSS; Invitrogen, Basel, Switzerland). At the same time, mock-infected cells were grown and handled under identical conditions and mixed with the infected cells at a ratio of 80% infected to 20% non-infected.

For infection, the following viruses were used: FPV (FPL/01, Intervet, UK), FCV (F9 strain, Intervet, UK), and FHV (Zurich 5-04, a Swiss isolate obtained from a cat suffering from a herpes keratitis). All viruses were tested for the absence of contaminations by other viruses and agents by PCR and RT-PCR specific for FHV, FCV, FPV, FCoV, FIV, FeLV, CDV, *A. phagocytophilum* and *E. canis*.

Preparation of the IFA slides: 7 µl of a suspension containing 1.5 X 10^6 cells per ml were placed on each well. The slides were air-dried, fixed in acetone for 10 min at -20°C, and stored at -20°C.

IFA testing: the sera were screened at a dilution of 1:20 in phosphate buffered saline (PBS; 2 mM NaH_2PO_4, 8 mM $NaHPO_4$, 0.15 M NaCl, pH 7.2). Incubation was carried out at 37°C for 60 min; after a washing step and air drying, FITC conjugate (rabbit anti cat IgG, H+L chain, Nordic Immunology, Tilburg, The Netherlands) diluted 1:40 in PBS was added for 60 min at 37°C. After a final wash (PBS), the slides were examined under a fluorescent microscope. The result was considered positive when specific fluorescence was detected in infected cells with a serum dilution of ≥ 1:20. Positive serum samples were titrated until end point on two-fold serial dilutions under conditions described above.

4.2.2 Detection of antibodies to FCoV

Antibodies to FCoV were detected by IFA as described previously (10). The sera were screened at a dilution of 1:20 in PBS. Any positive serum sample was titrated to dilutions of 1:25, 1:100, 1:400 and 1:1600.

4.2.3 Detection of antibodies to FeLV

Antibodies to FeLV p45, the unglycosylated form of gp70, were measured by enzyme-linked immunosorbent assay (ELISA) as described previously (10). Samples yielding > 25% of the positive control, run with each assay, were considered positive. Additionally, the questionable sera (> 15% and ≤ 25% of the positive control) were

examined for the presence of antibodies to FeLV by Western blot (14). Samples containing antibodies to gp70, p58, p27, p15 and p12 were judged positive (15).

4.2.4 Detection of antibodies to FIV

To detect antibodies to the lion lentivirus, domestic cat FIV Z2 (Zurich 2 strain) was used as antigen. Antibodies were detected by the Western blot technique as described previously (14). The presence of both the p24 band and p15 band and the presence of p24 band alone were interpreted as a positive result.

The sera were also tested by ELISA using recombinant FIV Z2 transmembrane glycoprotein (designated TM ELISA) as described elsewhere (3). Samples that reached an OD value > 50% of the positive control were considered positive.

4.2.5 Detection of antibodies to CDV

Antibodies to CDV were detected by IFA and immunohistochemistry using antigens produced as described (5). Briefly, for IFA the following conditions were used: Confluent VERO cell monolayers grown under the same conditions as described for CRFK were infected with CDV (Onderstepoort strain). The cells were removed from the bottom of the 75 cm^2 cell culture flask using 1 ml of Trypsin-EDTA (Invitrogen; 2.5 g/L of Trypsin and 0.38 g/L of EDTA in HBSS) when 30% of the cells were infected. These cells were used for IFA under conditions identical to those described above for FPV, FCV, FHV. Additionally, in every second well 100% non-infected cells were placed in order to have an external negative control. All sera were screened at a dilution of 1:80. Positive serum samples were titrated up to 1:320. Positive and questionable samples were confirmed using immunohistochemistry.

Immunohistochemistry: VERO cells were grown on glass cover slips and infected with CDV (Onderstepoort strain and A75/17-CDV, respectively). When cytopathic effect reached 30%, the cells were washed 3 times with Tris-buffered saline (TBS; Sigma) and blocked with normal goat serum (diluted 1:20 in TBS) for 20 min. The serum dilutions (1:200) in TBS were added without washing. After incubating for 2 h, the cover slips were washed and a peroxidase-conjugated goat anti-cat IgG preparation (Jackson ImmunoResearch Laboratories, West Baltimore Pike, Pennsylvania, USA) at a dilution of 1:1,000 was added and incubated for 1 h at 37°C. After washing thoroughly with TBS the substrate (1.85 mM 3,3’ Diaminobenzidine Tetrahydrochloride dissolved in TBS, 0.24‰ H_2O_2) was added. The enzymatic reaction was stopped after 10 min at 37°C.

4.2.6 Detection of antibodies to *Toxoplasma gondii*

96-well microtiter plates (Nunc Maxisorp, Nunc, Denmark) were coated with Toxoplasma antigen (Virion Ltd, Rueschlikon, Switzerland) at a concentration of 5.5 µg/ml carbonate/bicarbonate buffer (0,1 M, pH 9.6) containing 0.02% NaN_3, overnight at 4°C.

Plates were washed three times with 0.9% NaCl/0.3% v/v Tween 20 and saturated for 30 min at 37°C with PBS (pH 7.2) containing 0.02% NaN_3, 0.05% bovine hemoglobin and 0.3% v/v Tween 20 (PBS-T). Sera were diluted 1:200 in PBS-T and 100 µl per well were used. After incubation for 1 h at 37°C plates were washed four times and 100 µl per well detection antibodies conjugated to alkaline phosphatase (goat anti feline IgG, Southern, 1:4,000 in PBS-T) were added. After 1 h at 37°C plates were washed four times and 100 µl of a 1 mg per ml solution of p-nitrophenyl phosphate (Sigma) in 0.05 M carbonate/bicarbonate buffer containing 1mM $MgCl_2$ (pH 9.8) were added to each well. After 15 min the reactions were stopped by addition of 50 µl of 3 M NaOH. Absorbance was measured at 405 nm. The procedure had been optimized with regard to antigen concentration and conjugate dilution in pre-experiments (data not shown). Samples reaching an OD value > 50% of the control serum were judged positive. The threshold was based on the mean OD value plus 3 standard deviations of 20 *Toxoplasma*-free domestic cats.

4.2.7 Detection of antibodies to Anaplasma phagocytophilum and Ehrlichia canis

Exposure to *A. phagocytophilum* and *E. canis* was tested by IFA at a 1:80 starting dilution of all sera. *E. canis* slides (VMRD, Inc. Pullman, Washington, USA) were used for the detection of antibodies to *E. canis*, and *E. equi* slides (VMRD) for the detection of antibodies to *A. phagocytophilum*, respectively. Positive sera were titrated and examined until end point on two-fold serial dilutions. Samples reaching a titer ≥ 1:80 were considered positive.

4.3 Quality control of antigen preparation, total nucleic acid extraction and PCR

All antigens used for the immunofluorescence assays (IFA) were tested by PCR or RT-PCR for absence of possibly contaminating antigens. To this end, aliquots of the

cell cultures or scrapings from the slides were tested for presence of FCV, FHV-1, FPV, FCoV, CDV, FIV, FeLV, *A. phagocytophilum* and *E. canis*.

For the extraction of nucleic acids from the aliquots, the MagNA Pure LC instrument (Roche Diagnostics AG, Rotkreuz, Switzerland) using the Total Nucleic Acid Isolation Kit (Roche Diagnostics AG) was used. Briefly, 140 µl of the aliquots or the scrapings from the slides were incubated at 40°C for 10 min in 300 µl lysis buffer. The purified nucleic acids were eluted in a final volume of 100 µl.

The extracted total nucleic acid samples were analyzed by one-tube real-time PCR using an automated fluorometer (TaqMan, ABI 7700, Applied Biosystems, Rotkreuz, Switzerland) for the presence of the agents of interest using the following primers or conditions: FCV: primers and probe sequences were slightly derived from those published (9) and kindly provided by C. Helps: forward 5'-GTTGGATGAACTACCCGCCAATC-3', reverse 5'-CATATGCGGCTCTGATGGCTTGAAACTG-3', probe 5'-TCGGTGTTTGATTTGGCCTG-3'. CDV was tested as described (7). Conditions were as published for FHV (30), FPV (17), FCoV (8, 17), FIV (1), FeLV (11), *A. phagocytophilum* (21) and *E. canis* (21).

4.4 Statistical methods

Frequencies were compared by the Pearson Chi square test and the actus randomization test (6) followed by Bonferroni correction (22). Fisher's exact test was used when a table had a cell with an expected frequency of less than 5 (SPSS, version 11.5). Differences were considered significant at $p < 0.05$.

5 Results

All results were analyzed with respect to habitat, sex and age of lions and year of blood collection if the respective information was available. Whenever a correlation between the results and the above mentioned parameters was found, it is stated; otherwise no information is given.

The overall prevalence of antibodies to selected feline and canine infectious agents in captive and free-ranging lions is compiled in Table 1. The seroprevalences according to geographical locations in free-ranging lions are shown in Table 2.

5.1 Prevalence of antibodies to FHV

Titers ranged from 20 to 320. A significant difference was found to be related to the origin of the samples ($p_{chi} < 0.0001$): 35 of 43 (81%) sera from free-ranging lions tested positive. In contrast, only 7 of 29 captive lions produced antibodies with reactivity to FHV ($p < 0.01$).

A positive FHV antibody status was significantly associated with antibodies to *T. gondii* ($p_{chi} < 0.001$) (Table 5).

5.2 Prevalence of antibodies to FCV

One captive animal had a low titer of 40. This serum also tested positive for antibodies to FHV.

5.3 Prevalence of antibodies to FPV

The titers ranged from 20 to > 10,240. Low titers (< 160) were detected in 8 lions, whereas high titers were found in 18 animals. A significant association was found with sex of the lions ($p_{chi} = 0.023$): 9 out of 17 samples of male lions test positive (53%). In contrast, only 7 out of 33 samples of lionesses were positive (21%).

5.4 Prevalence of antibodies to FCoV

Among the 72 lions, 5 were FCoV antibody positive with titers of 25 (2 free-ranging and 3 captive lions) while 1 free-ranging animal had a titer of 100.

5.5 Prevalence of antibodies to FeLV

All lions tested negative for the presence of antibodies to FeLV. Out of the 72 serum samples, 7 samples reached levels between 15 to 22% of the positive control when

tested by ELISA. When tested by Western blot, none yielded the typical pattern of FeLV antibodies; all sera were therefore judged negative.

5.6 Prevalence of antibodies to FIV

The results of the serum samples tested for antibodies to FIV are summarized in Table 3.

A total of 53% of the free-ranging and 21% of the captive lions were FIV positive.

FIV positivity was significantly associated with free-ranging ($p < 0.05$) and the age of the lions ($p_{chi} = 0.006$): 16 of 34 adult animals showed antibodies to FIV (47%). In contrast, only 1 out of 15 juveniles (< 4y) tested positive (7%). From 32 animals, the age was unknown. A significantly different prevalence was found when the data were analyzed according to the origin of the lions sampled ($p_{chi} = 0.007$): 22 of 42 free-ranging lions tested positive (52%) whereas only 6 of 29 captive lions had a positive test result (21%). Regardless of the origin, the virus was endemic in every region sampled with the exception of two groups of captive lions and one group of 3 free-ranging lions inhabiting Hwange National Park.

There were some significant associations between antibodies to FIV and antibodies to FHV and *T. gondii* (Table 4). Antibodies to FHV and to *T. gondii* were significantly associated with FIV seropositivity ($p_{chi} = 0.029$).

None of the samples tested in TM ELISA was considered positive (> 50% of positive control).

5.7 Prevalence of antibodies to CDV

Three out of the five CDV positive sera were confirmed to be positive by immunohistochemistry (4%). The 3 lions were free-ranging and were sampled in Matusadona National Park ($p_{chi} < 0.001$) (Table 2).

5.8 Prevalence of antibodies to Toxoplasma gondii

A significant association was found with the origin of the serum samples ($p_{chi} < 0.0001$): the majority of the free-ranging lions (86%) showed antibodies with reactivity to *T. gondii*. In contrast, in captive animals the prevalence of antibodies to *T. gondii* was 31%. There was a significant positive correlation between antibodies to *T. gondii* and antibodies to FHV ($p_{chi} < 0.001$). A positive *T. gondii* status was significantly associated with absence of antibodies to both FPV ($p_{chi} = 0.005$) (Table 5).

5.9 Prevalence of antibodies to Anaplasma phagocytophilum

Among the 81 lions, 6 sera showed specific fluorescence to *A. phagocytophilum* (7%). The titers ranged from 80 to 640. All positive animals were free-ranging.

5.10 Prevalence of antibodies to Ehrlichia canis

Antibodies to *E. canis* were detected in none of the 72 serum samples tested.

6 Discussion

In the present study serum samples collected from free-ranging and captive lions from Zimbabwe were analyzed for the presence of antibodies to various infectious agents. The study was undertaken as only limited information was available to date on the infectious disease status of Zimbabwean lions. In past studies major differences had been observed in the prevalence of numerous diseases in lions originating from East Africa, West Africa and South Africa (1, 10, 27-29) (Table 6).
An ideal basis for any seroprevalence study would be a serum collection carried out within a defined, short period of time in order to establish sound information on a given time point. The present study is based on serum samples collected opportunistically over a time period of twelve years from lions immobilized for various reasons such as translocations and radio-collaring. Therefore the present study was not done under ideal conditions but allows an assessment of the infections studied.

6.1 Feline herpes virus

The overall prevalence of FHV antibodies of 81% in free-ranging lions is in good agreement with the situation in the Serengeti National Park, the Ngorongoro Crater, the Lake Manyara region, the Kruger National Park, and the Etosha National Park; in all these areas the prevalence of FHV was generally high (Table 6). The observation that FHV prevalence is higher in free-ranging than in captive lions may be explained by the fact that captive animals are generally kept isolated, thus there is reduced contact.

6.2 Feline calici virus

No FCV antibodies were found in lions with exception of one captive animal., Therefore, FCV is of no importance in free-ranging lions in Zimbabwe. This is in agreement with the situation found in the Ngorongoro Crater, Lake Manyara region, the Kruger Park, and the Etosha National Park, where FCV was undetectable (10, 27, 29), but in contrast to that in the Serengeti National Park, where the FCV prevalence was found to be 67% (10) (Table 6). No explanation can be offered for the high prevalence of antibodies to FCV in the Serengeti and the low prevalence in Zimbabwe. Certainly, it cannot be explained by different assays as in both studies the same antigen was used.

6.3 Feline parvo virus

FPV antibodies were readily detected in free-ranging lions, with a prevalence of 23%. Thus, FPV infection seems to be of considerable importance. This is in contrast to the findings in Etosha where the prevalence was found to be zero (29), but is in agreement with the situation in East and South Africa with prevalences between 27 and 84% (10, 27, 29) (Table 6). The observation that FPV antibodies were significantly associated with the male sex might be explained by the fact that young adult male lions usually have to leave their pride and will wander around to a greater extent than their female family members thus increasing the chance of exposure to the virus.

6.4 Feline corona virus

The prevalence of 7% found in 43 free-ranging Zimbabwean lions was not surprising. It was in the same range as in the Etosha National Park, though the prevalence in the Kruger National Park was 0% (27). However, this is significantly lower than in the lion populations in East Africa. In the earlier paper (10), we had speculated that the difference in the FCoV prevalence in East and South Africa might be due to assay conditions, which were not known for reports emanating in South and West Africa (27, 29). The test used for the present study was identical to the one used previously in East Africa (10) so that a difference can hardly be explained by varying assay conditions. In view of the fact that the same assay found a high prevalence of FCoV antibodies in East Africa but a low in Zimbabwe, it appears likely that indeed the prevalence of FCoV antibodies in lions in West and South Africa is less than in Tanzania and in Kenya (Table 6). This does not rule out the possibility that different subtypes of FCoV may be present in these different locations.

6.5 Feline leukaemia virus

None of the lions in the present study tested positive for FeLV antibody, which is identical to all other lion populations studied so far. We conclude that FeLV infection also in Zimbabwean lions does not play a major role.

6.6 Feline immunodeficiency virus

In the present study 52% of the free-ranging animals were positive by WB using domestic cat FIV. This included samples which produced only a single p24 band in Western blots and samples that recognized several major bands. This prevalence is

significantly lower than that found in East Africa and in the Kruger National Park, where it was higher than 90% (Table 6). In the present study, we considered as positive also those samples that recognized only the p24 band and with this may have overestimated the true prevalence. In man, it has been shown that antibodies reacting with HIV p24 can be induced not only by HIV but also by influenza infection based on a short identical amino acid sequence present on HIV p24 and influenza virus. In the diagnosis of HIV infection, it is a well known phenomenon that occasionally antibodies to p24 are detected in individuals that are not infected with HIV. These reactions can be explained by the presence of antibodies induced by peptides of unknown infectious agents which are similar or identical to peptides of HIV p24 (2, 25). A similar cross reactivity could also exist in lions. From this it must be concluded that the true prevalence of lentivirus infection in Zimbabwean lions is indeed much lower than in East or South Africa, or that a virus is present in this population which is more distantly related from the domestic cat FIV than the lentivirus present in East Africa or in the Kruger National Park.

As in previous studies, the Zimbabwean lions showed a significant association of FIV infection with age. This most likely reflects the fact that exposure to FIV increases with age (19). Interestingly, lions kept in captivity showed a significantly lower prevalence of FIV than those in the wild. It appears that the lower prevalence is not associated with younger age as there was no significant difference in the age of the captive versus free-ranging lions. An explanation could be that lions in captivity generally would fight less than in the wild because they are living in smaller groups and have no territory to defend against intruders, or that fact that the populations are closed. The strong association between FIV, FHV and *T. gondii* infection is most likely due to the differences in the prevalences of both infections in captive and free-ranging lions and therefore do not represent causal associations.

6.7 Canine distemper virus

The prevalence of antibodies to CDV was low, which is similar to the situation in the Serengeti lion population shortly before a severe outbreak of CDV infection in 1994 (19, 23). While in East Africa it was clearly shown that the prevalence of antibodies to CDV varied over time, the prevalence of antibodies to CDV in Zimbabwean lions seems to have remained low over an extended period of time (12 years of sampling). Positive IFA results were confirmed by the highly specific immunohistochemistry (5). As only three out of five IFA positive samples were clearly confirmed by

immunohistochemistry, the true prevalence of antibodies to CDV must indeed be very low in the Zimbabwean lions. Generally, the areas where free-ranging Zimbabwean lions roam are less populated by humans and dogs than in East Africa, which may account for the difference in CDV seropositivity. Alternatively, because Rinderpest, which has close cross-reactivity to CDV (26), has been absent from Zimbabwe since 1900, and vaccination has never been introduced in the country, the possibility of Rinderpest virus inducing seroconversion detectable by CDV antigen in lions in Zimbabwe does not exist. The 3 positive lions originated from the Matusadona National Park. This observation is surprising because more dog contacts would have been expected in the Mola/Bumi area.

6.8 Toxoplasma gondii

Antibodies to *T. gondii* were highly prevalent. This observation is in agreement with previous studies from South Africa (4). The significant difference between the test results of free-ranging (86% positive) and captive lions (32% positive) could be explained by differences in the diet. Captive lions are usually fed carcass material from domestic cattle.

6.9 Anaplasma phagocytophilum

Although interesting, the 14% prevalence of antibodies to *A. phagocytophilum* in free-ranging lions is not a surprise, because many genera of ticks are present in Zimbabwe and will feed on lions. *A. phagocytophilum* is known to affect many species worldwide (7, 12, 16, 24). Antibodies to *A. phagocytophilum* have not been previously reported, so the high titers recorded in this study cannot be said to be unusual. The pathogenicity of *A. phagocytophilum* for lions is unknown.

6.10 Ehrlichia canis

No lion tested positive for *E. canis*. This could mean that either *E. canis* does not infect lions or if it does it is highly pathogenic and leads to rapid disease progression and death thereby eliminating all animals that might have been infected. Interestingly, we have recently identified a wild puma in Brazil with a very high titer to *E. canis* (7) . This suggests that also lions may be susceptible for *E. canis* infection. E. canis infection is commonly diagnosed in dogs in Zimbabwe.

This study has shown that lions in Zimbabwe may be infected with a large variety of infectious agents which vary in prevalence in different areas, situations and age groups. Although it is unknown if any of these agents can affect the population dynamics of lions in Zimbabwe, every translocation of animals of this species and introduction into another population should be carefully considered in the light of these findings. It is recommended that the serological status of animals destined for translocation be assessed whenever possible.

7 Figures and Tables

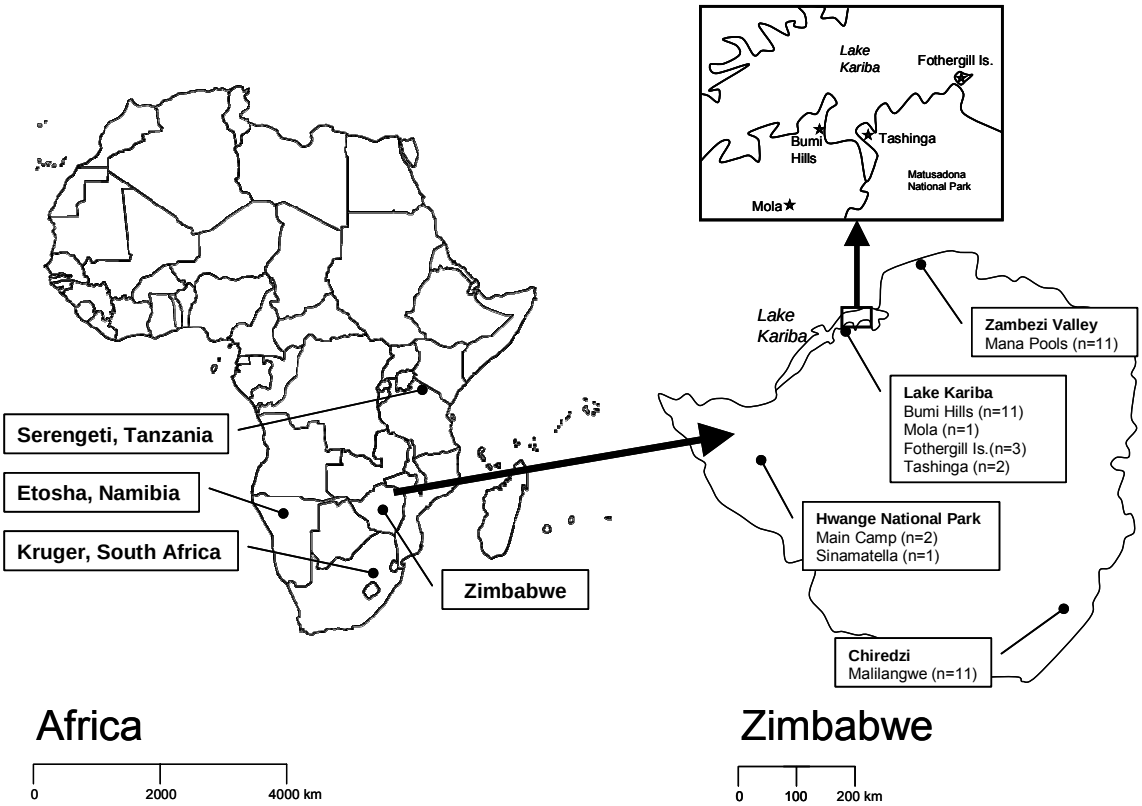

FIG. 1. Maps of Africa and of Zimbabwe. The free-ranging lions inhabited areas next to Lake Kariba, Mola (n=1), Bumi Hills (n=11), Fothergill Is. (n=3), Tashinga (n=2), also Chiredzi, Malilangwe (n=11), Zambezi Valley, Mana Pools (n=11), and Hwange National Park, Main Camp (n=2), Sinamatella (n=1. Numbers in parenthesis indicate the numbers of samples collected in the respective areas. In addition, Etosha National Park in Namibia, Kruger National Park in South Africa and Serengeti National Park in Tanzania are indicated.

TABLE 1. Comparison of the seroprevalence of different infectious agents in free-ranging and captive lions.

Infectious Agent	Free-ranging			Captive			Statistics[g]	
	N pos./N	prev.	(95%-CI)	N pos./N	prev.	(95%-CI)	chi^2	P[h]
FHV[a]	35/43	81%	(67-92)	7/29	24%	(10-44)	23.3	<0.01
FCV[a]	0/43	0%	(0-8)	1/29	3%	(0-18)	[i]	
FPV[a]	10/43	23%	(12-39)	11/29	38%	(21-58)	1.8	n.s.
FCoV[a]	3/43	7%	(1-19)	3/29	10%	(2-27)	[i]	
E. canis[b]	0/43	0%	(0-8)	0/29	0%	(0-12)	[i]	
A. phagocytophilum[b]	6/43	14%	(5-28)	0/29	0%	(0-12)	[i]	
CDV[c]	3/43	7%	(1-19)	0/29	0%	(0-12)	[i]	
FeLV (gp70)[d]	0/43	0%	(0-8)	0/29	0%	(0-12)	[i]	
T. gondii[e]	37/43	86%	(72-95)	9/29	31%	(15-51)	22.7	<0.01
FIV[f]	22/42	52%	(36-68)	6/29	21%	(8-40)	7.2	<0.05

a IFA titer ≥ 20.
b IFA titer ≥ 80.
c IFA titer ≥ 80 and confirmed using immunohistochemistry.
d ELISA OD > 15% of pos. control and Western blot positive.
e ELISA OD > 50% of pos. control.
f Western blot: positive and questionable results were taken together and considered positive for the purpose of this table.
g Actus randomization test
h Bonferroni corrected P-values
i less than 10 positive animals => no statistical analyses

TABLE 2. Comparison of the seroprevalence of FHV, FPV, FCoV, FIV, A. phagocytophilum, T. gondii and CDV in free-ranging lions from different parts of Zimbabwe.

Area	No. of sera positive/no. tested for:													
	FHV[a]		FPV[a]		FCoV[a]		FIV[b]		*A. phag.*[c]		*T. gondii*[d]		CDV[e]	
Zambezi Valley *Mana Pools*	9/11	(82%)	5/11	(45%)	2/11	(18%)	8/11	(73%)	2/11	(18%)	8/11	(73%)	0/11	(0%)
Chiredzi *Malilangwe*	9/11	(82%)	3/11	(27%)	1/11	(9%)	7/11	(64%)	1/11	(9%)	10/11	(91%)	0/11	(0%)
Lake Kariba *Bumi Hills*	8/11	(73%)	1/11	(9%)	0/11	(0%)	4/10	(40%)	2/11	(18%)	10/11	(91%)	0/11	(0%)
Lake Kariba *Mola*	1/1		0/1		0/1		1/1		0/1		1/1		0/1	
Matusadona *Fothergill Is.*	3/3		1/3		0/3		2/3		1/3		3/3		2/3	
Matusadona *Tashinga*	2/2		0/2		0/2		0/2		0/2		2/2		1/2	
Hwange *National Park*	2/3		0/3		0/3		0/3		0/3		2/3		0/3	

a IFA titer ≥ 20.
b Western blot: positive and questionable results were taken together and considered positive for the purpose of this table (more data will be presented separately).
c A. phagocytophilum.
d ELISA OD > 50% of pos. control.
e IFA titer ≥ 80 and confirmed by immunohistochemistry.

TABLE 3. Comparison of the results of the FIV-Western blot in free-ranging and captive lions in Zimbabwe.

	FIV[a]		
	p24/p15	p24	Negative
Free-ranging	4/42 (10%)	18/42 (43%)	20/42 (48%)
Captive	2/29 (7%)	4/29 (14%)	23/29 (79%)

a Western blot (n = 80, 1 sample not sufficient amount of serum).

TABLE 4. Relationship between antibodies to FIV and antibodies to FHV and T. gondii.

FIV antibody status	Total (n=71)				Free-ranging (n=43)				Captive (n=29)			
	No. of sera positive or negative for antibody to:				No. of sera positive or negative for antibody to:				No. of sera positive or negative for antibody to:			
	FHV		*T. gondii*		FHV		*T. gondii*		FHV		*T. gondii*	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Pos.	22	6	22	6	20	2	21	1	2	4	1	5
Neg.	20	23	23	20	15	5	15	5	5	18	8	15
	$p_{chi} = 0.007$		$p_{chi} = 0.032$		NS		NS		NS		NS	

TABLE 5. Relationship between antibodies to T. gondii and antibodies to FHV, FPV and FCV (n = 81).

T. gondii antibody status	Total (n=72)				Free-ranging (n=43)				Captive (n=29)			
	No. of sera positive or negative for antibody to:				No. of sera positive or negative for antibody to:				No. of sera positive or negative for antibody to:			
	FHV		FPV		FHV		FPV		FHV		FPV	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Pos.	39	7	9	37	34	3	7	30	5	4	2	7
Neg.	3	23	12	14	1	5	3	3	2	18	9	11
	$p_{chi} < 0.001$		$p_{chi} < 0.001$		$P_{Fisher} < 0.001$		NS		$P_{Fisher} = 0.016$		NS	

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